Vol.20, No.2, Page 517- 529, 2026 http://dx.doi.org/10.59628/jchm.v20i2.2755 ISSN 2958-7476 JMCH

Sana'a University Journal of Medical and Health Sciences

Protective Role of Amlodipine Against Cyclophosphamide- and Acetaminophen-Induced Nephrotoxicity: Modulation of Gamma-Glutamyl Transpeptidase and Oxidative Stress Pathways in Rats.

Majdi Ali Al-Somat[1,4] *, Hassan Mohammed Al-Mahbashi[2] and Afif Saeed Al-Nabehi[3]

[1]**Department of Pharmacology &Therapeutics, Faculty of Medicine and Health Sciences, Sana'a University, Sana'a Yemen. ,**
[2]**Department of Forensic Medicine and Clinical Toxicology, Faculty of Medicine and Health Sciences, Sana'a University, Yemen,**
[3]**Department of Medicine, Faculty of Medicine, Sana'a University, Sana'a Yemen,**
[4]**Al-Afaq College for Medical and Technical Sciences, Sana'a, Yemen.**

*Corresponding author: M.Al-Somat@su.edu.ye

Abstract

Background:
Cyclophosphamide (Cyclo) is a widely used chemotherapeutic agent that induces nephrotoxicity via oxidative stress and dysregulation of gamma-glutamyl transpeptidase (GGT). Acetaminophen (Aceta) may exacerbate these effects by increasing the oxidative burden, whereas amlodipine (Amlo), a calcium channel blocker with antioxidant properties, may confer nephroprotection.

Objective :
This study aimed to evaluate the protective role of amlo against cyclophosphamide- and acetaminophen-induced nephrotoxicity, focusing on GGT modulation and oxidative stress markers.

Methods:
Fifty-six male albino rats were randomly assigned to seven groups: control, cyclophosphamide (Cyclo), amlodipine (Amlo), acetylsalicylic acid (Aceta), Cyclo + Amlo, Cyclo + Aceta, and Cyclo + Amlo + Aceta. Treatments were administered for 14 days, with a single intraperitoneal dose of cyclo (200 mg/kg) on day 10. Renal function (BUN, serum creatinine, and urea), renal GGT activity, and oxidative stress parameters (GSH, GPx, MDA, CAT, NO, and SOD) were assessed at the study endpoint. Histopathological analysis, including glomerular capillary space measurements, was also performed.

Results:
Cyclo and Aceta significantly increased renal GGT and MDA levels and reduced antioxidant enzyme levels (GSH, GPx, CAT, NO, and SOD), confirming oxidative nephrotoxicity. Amlo alone preserved renal function and antioxidant status. Co-administration of amlodipine with cyclophosphamide markedly attenuated oxidative stress, restored antioxidant enzyme activity, and improved histological integrity. Conversely, the combination of Cyclo and Aceta exacerbated oxidative and structural renal damage. Notably, the triple combination (Cyclo + Amlo + Aceta) demonstrated partial protection, highlighting the dominant nephroprotective effect of amlodipine.

Conclusion:
Amlodipine demonstrated a protective effect against cyclophosphamide- and acetaminophen-induced nephrotoxicity by reducing oxidative stress and preserving renal structure. In contrast, acetaminophen exacerbated renal injury. Cyclophosphamide-induced nephrotoxicity may not be detected by conventional renal function tests but is evident at the oxidative and histopathological levels. These findings highlight the importance of oxidative stress biomarkers, including GGT, and support amlodipine as a potential renoprotective strategy; however, caution is advised when it is combined with acetaminophen.

Article Info

Keywords:
Cyclophosphamide, Acetaminophen, Amlodipine, Nephrotoxicity, Gamma-Glutamyl Transpeptidase, Oxidative Stress.

Article History:
Received: 17-January-2026,
Revised: 24-March-2026,
Accepted: 08-April-2026,
Published: 28 April 2026.

1. INTRODUCTION

Gamma-glutamyl transpeptidase (GGT) is a membrane-bound glycoprotein that plays a pivotal role in the maintenance of glutathione (GSH) and cysteine homeostasis. Widely distributed across multiple organs, this microsomal enzyme contributes to glutamyl group transfer, protein synthesis, and detoxification [1]. Elevated serum and urinary GGT levels have been clinically associated with chronic kidney disease (CKD) and acute kidney injury (AKI), underscoring their importance as biomarkers of renal health [2, 3].

Drug-induced nephrotoxicity remains a major cause of morbidity and a leading reason for the withdrawal of medications from clinical use [4–7]. Conventional markers such as serum creatinine and blood urea nitrogen (BUN) are limited in sensitivity, as they typically reflect changes in the glomerular filtration rate only after substantial renal damage has occurred [8–10].

Cyclophosphamide (Cyclo), a potent cytotoxic alkylating agent, is well known for its nephrotoxic and hepatotoxic effects [11, 12]. Its metabolism generates reactive oxygen species (ROS) and the toxic metabolite acrolein, which induce oxidative stress, deplete glutathione (GSH), and activate inflammatory pathways [13–17]. Although GGT activity initially increases to support GSH resynthesis, prolonged activation can paradoxically aggravate tubular injury through redox cycling and lipid peroxidation [16–18]. Similarly, acetaminophen (Aceta) toxicity arises when metabolic pathways are saturated, leading to the accumulation of N-acetyl-p-benzoquinoneimine (NAPQI), depletion of GSH, and GGT-mediated oxidative imbalance [19–21].

In contrast, amlodipine (Amlo), a dihydropyridine calcium channel blocker, has demonstrated nephroprotective properties beyond its antihypertensive effects [19, 20]. Evidence suggests that amlodipine mitigates renal injury by suppressing pro-inflammatory cytokines (TNF-α and IL-6), stabilizing mitochondrial function, and exerting strong antioxidant effects [22, 23]. In models of cyclo-induced toxicity, amlo has been shown to inhibit excessive GGT activation and preserve intracellular GSH levels [19, 24].

Therefore, the present study aimed to evaluate the protective effects of amlodipine against nephrotoxicity induced by cyclophosphamide and acetaminophen, with particular emphasis on GGT regulation and oxidative stress biomarkers.

2. MATERIALS AND METHODS

2.1. Drugs

Cyclophosphamide was procured from GLS Pharma Ltd. (India). Amlodipine was supplied by Denk Pharma GmbH. KG (Germany). Acetaminophen was obtained from Global Pharma (Dubai, UAE).

2.2. Animals

Fifty-six adult male albino rats (150–250 g) were obtained from the Animal House of the Department of Biology, Sana'a University. Animals were housed under standard laboratory conditions with controlled temperature and humidity and maintained on a 12 h light/dark cycle. The rats had free access to a standard pellet diet and tap water ad libitum throughout the study. All procedures were conducted in accordance with the National Institutes of Health (NIH) guidelines for the care and use of laboratory animals and were approved by the Institutional Animal Care and Use Committee (IACUC), Sana'a University (Approval Code No. 25/8-5).

2.3. Experimental design

Fifty-six rats were randomly allocated to seven groups (n = 8 per group):

Group I (Control): Normal saline (2.5 mL/kg, i.p.) once daily for 14 days. Group II (Cyclo): Single intraperitoneal injection of cyclophosphamide (200 mg/kg) on day 10. Group III (Amlo): Oral amlodipine (5 mg/kg/day) for 14 days. Group IV (Aceta): Oral acetaminophen (75 mg/kg/day) for 14 days. Group V (Cyclo + Amlo): Oral amlodipine (5 mg/kg/day) for 14 days plus a single intraperitoneal dose of cyclophosphamide (200 mg/kg) on day 10. Group VI (Cyclo + Aceta): Oral acetaminophen (75 mg/kg/day) for 14 days plus a single intraperitoneal dose of cyclophosphamide (200 mg/kg) on day 10. Group VII (Cyclo + Amlo + Aceta): Oral amlodipine (5 mg/kg/day) and acetaminophen (75 mg/kg/day) for 14 days, plus a single intraperitoneal dose of cyclophosphamide (200 mg/kg) on day 10.

Doses and treatment regimens were selected based on preliminary experiments and previously published studies [25–27].

2.4. Sample collection and storage

At the end of the experimental period, the rats were anesthetized with sodium thiopental (40 mg/kg, i.p.; Panpharma, France). Adequate anesthesia was confirmed by the absence of pedal withdrawal reflex and stable respiration [28]. Approximately 3 mL of blood was collected via cardiac puncture with a 23-gauge needle. Animals were then euthanized with a lethal dose of sodium thiopental (150 mg/kg, i.p.), followed by bilateral thoracotomy to ensure irreversible cessation of cardiac activity, in compliance with the international euthanasia guidelines [29–31].

Blood samples were allowed to clot and centrifuged to

obtain serum for biochemical analyses. The kidneys were excised, rinsed with phosphate-buffered saline, and divided into two portions: one was fixed in 10% neutral-buffered formalin for histopathology, and the other was homogenized in cold saline, centrifuged, and stored at -80 ℃ for biochemical and oxidative stress assays.

At the end of the experimental period, the rats were anesthetized with sodium thiopental (40 mg/kg, i.p.; Panpharma, France). Adequate anesthesia was confirmed by the absence of pedal withdrawal reflex and stable respiration [28]. Approximately 3 mL of blood was collected via cardiac puncture with a 23-gauge needle. Animals were then euthanized with a lethal dose of sodium thiopental (150 mg/kg, i.p.), followed by bilateral thoracotomy to ensure irreversible cessation of cardiac activity, in compliance with the international euthanasia guidelines [30–32].

Blood samples were allowed to clot and centrifuged to obtain serum for biochemical analyses. The kidneys were excised, rinsed with phosphate-buffered saline, and divided: one portion was fixed in 10% neutral-buffered formalin for histopathology, and the other was homogenized in cold saline, centrifuged, and stored at -80 ℃ for biochemical and oxidative stress assays.

2.5. Biochemical analysis of kidney function

Serum urea, creatinine, and blood urea nitrogen (BUN) levels were measured using commercial colorimetric kits (Biosystems Diagnostics, Spain; catalogue nos. REF 11698 and 11536), following the manufacturer's protocols. The absorbance was recorded using a semi-automated spectrophotometer (Rayto RT-9200, Germany).

2.6. Assessment of oxidative stress/antioxidant parameters of renal tissue

Renal oxidative stress and antioxidant status were assessed using commercial kits (Bio-Diagnostic, Giza, Egypt; catalogue nos. GR 25 11, GP 25 24, MD 25 29, CA 25 17, NO 25 33, SD 25 21).

Reduced glutathione (GSH) levels were determined using the method described by Beutler et al. (1963) [33], glutathione peroxidase (GPx) activity according to Paglia and Valentine (1967) [34], malondialdehyde (MDA) content following Ohkawa et al. (1979) [35], catalase (CAT) activity as described by Aebi (1984) and Fossati et al. (1980) [36, 37], nitric oxide (NO) levels according to Montgomery (1961) [38], and superoxide dismutase (SOD) activity using the method described by Nishikimi et al. (1972) [39]. The absorbance was measured at 405, 340, 534, 510, 540, and 560 nm.

2.7. ELISA assay for renal GGT activity

Renal GGT activity was quantified using a commercial ELISA kit (Elabscience Biotechnology Inc., Wuhan, China; catalogue no. E.BC.K126.M). The assay measures the enzymatic transfer of the γ-glutamyl group from L-γ-glutamyl-p-nitroanilide to an acceptor, releasing p-nitroaniline. The color intensity was measured at 405 nm and was directly proportional to the GGT activity.

2.8. Histopathological study

Kidneys fixed in 10% neutral-buffered formalin were processed using standard paraffin embedding techniques. Sections (4–5 μm) were stained with periodic acid–Schiff (PAS) following Bancroft & Gamble (2008), Wherle et al. (2024) [40, 41], and Alshubaily & Jambi (2022) [42]. Histopathological evaluation was performed using a light microscope (Olympus BX53, Japan) at 400× magnification to assess tubular degeneration, glomerular damage, and interstitial inflammation.

2.9. Statistical analysis

Data were analyzed using GraphPad Prism (version 8.4.2). Normality was assessed prior to the analysis. Results are expressed as the mean ± standard deviation (SD). Group differences were evaluated using one-way ANOVA, followed by Tukey's post hoc test. Statistical significance was set at $P < 0.05$.

3. RESULTS

Table (1) shows that cyclophosphamide (Cyclo) administration did not induce significant nephrotoxicity, as no statistically significant changes were observed in blood urea nitrogen (BUN), serum creatinine (SC), or urea levels compared to the control group. Specifically, BUN levels were 13.88 ± 2.75 mg/dL in the Cyclo group versus 15.50 ± 1.06 mg/dL in the control group, serum creatinine levels were 1.4 ± 0.18 mg/dL versus 1.10 ± 0.10 mg/dL, and urea levels were 30.00 ± 5.47 mg/dL versus 32.87 ± 1.95 mg/dL, respectively. Similarly, treatment with amlodipine (Amlo) or acetaminophen (Aceta) alone did not significantly alter renal biomarkers, with BUN, serum creatinine, and urea levels remaining comparable to those of the control group.

In the interaction groups, co-administration of amlodipine with cyclophosphamide did not significantly affect the renal function parameters compared with cyclo treatment

Table[1]: Effects of Cyclo, Amlo, and Aceta individually or in combination on Blood Urea Nitrogen (BUN), Serum Creatinine (SC), and Urea.

Groups/ Tests	BUN (mg/dL)	SC (mg/dL)	Urea (mg/dL)
(G1) Control	15.50 ± 1.06	1.10 ± 0.10	32.87 ± 1.95
(G2) Cyclo	13.88 ± 2.75	1.40 ± 0.18	30 ± 5.47
(G3) Amlo	14.37 ± 2.50	1.01 ± 0.22	32.87 ± 4.96
(G4) Aceta	16.00 ± 2.00	1.23 ± 0.16	34.25 ± 4.89
(G5) Cyclo + Amlo	13.75 ± 3.49	1.20 ± 0.15	27.63 ± 8.58
(G6) Cyclo + Aceta	$9.37 \pm 1.68^{*/\#}$	1.31 ± 0.28	$20.25 \pm 3.53^{*/\#}$
(G7) Cyclo + Amlo + Aceta	12.13 ± 3.83	$1.50 \pm 0.29^{*}$	27.25 ± 7.17

► *Data expressed as mean ± SD. Statistical analysis carried out by Ordinary one − way ANOVA followed by Tukey multiple comparisons test.*
► $*$*Significantly different with respect to control* ($P < 0.05$), #*Significantly different with respect to cyclophosphamide* ($P < 0.05$), *Blood urea nitrogen* (*BUN*), *Serum Creatinine* (*SC*) *and urea.*

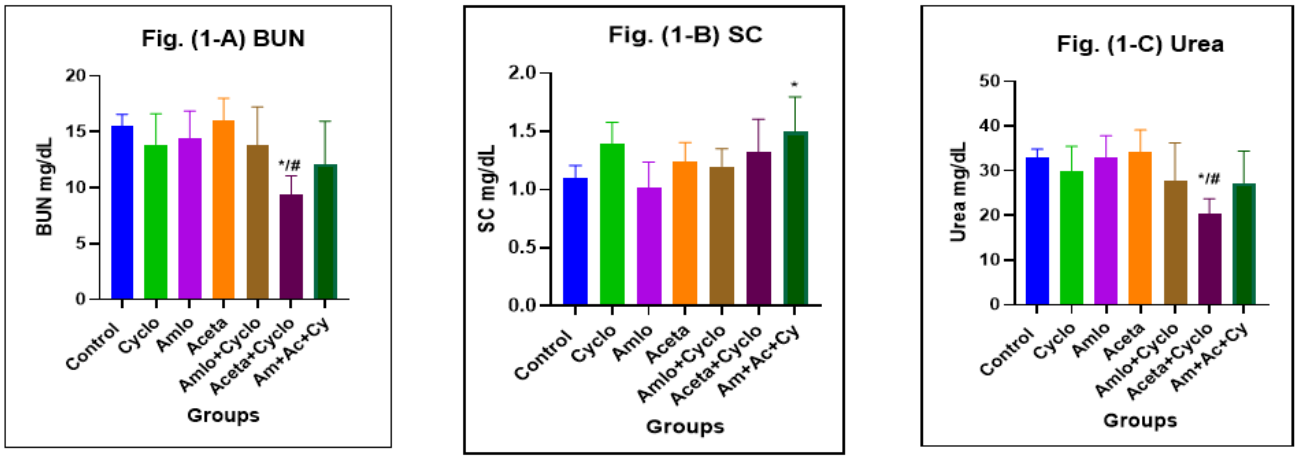

Figure 1. (A,B,C) Effects of Cyclo, Amlo, and Aceta individually or in combination on Blood Urea Nitrogen (BUN), Serum Creatinine (SC), and Urea.

alone, as evidenced by comparable BUN (13.75 ± 3.49 vs. 13.88 ± 2.75 mg/dL), serum creatinine (1.20 ± 0.15 vs. 1.40 ± 0.18 mg/dL), and urea (27.63 ± 8.58 vs. 30.00 ± 5.47 mg/dL) levels. In contrast, co-administration of acetaminophen with cyclophosphamide resulted in a significant reduction in BUN (9.37 ± 1.68 mg/dL) and urea levels (20.25 ± 3.53 mg/dL) compared with both the control and Cyclo groups (*P < 0.05, #P < 0.05), whereas serum creatinine levels remained unchanged (1.31 ± 0.28 mg/dL).

The triple combination (Cyclo + Amlo + Aceta) did not produce significant changes in BUN (12.13 ± 3.83 mg/dL) or urea levels (27.25 ± 7.17 mg/dL) compared with the Cyclo group; however, serum creatinine levels were significantly increased relative to the control group (1.50 ± 0.29 vs. 1.10 ± 0.10 mg/dL, *P < 0.05).

Building on these oxidative stress findings, Table (2) demonstrates that cyclophosphamide (Cyclo) administration induced significant oxidative alterations in the kidney tissue, as evidenced by a significant increase in gamma-glutamyl transpeptidase (GGT) activity (1.32 ± 0.34 vs. 0.80 ± 0.16 U/g) and malondialdehyde (MDA) levels (70.42 ± 10.38 vs. 31.37 ± 10.05 nmol/g) compared with the control group (*P < 0.05). In contrast, tissue nitric oxide (NO) levels were not significantly altered following cyclo treatment (3.09 ± 0.33 vs. 3.57 ± 0.23 μmol/L).

Administration of amlodipine (Amlo) alone did not produce significant oxidative stress, as GGT activity (0.71 ± 0.09 U/g), MDA (27.19 ± 13.14 nmol/g), and NO (3.52 ± 0.54 μmol/L) levels showed no significant differences compared to the control group. Conversely, acetaminophen (Aceta) alone exhibited intrinsic oxidative toxicity, significantly increasing GGT activity (1.36 ± 0.18 vs. 0.80 ± 0.16 U/g) and MDA levels (128.4 ± 22.11 vs. 31.37 ± 10.05 nmol/g) and significantly reducing NO levels (2.75 ± 0.72 vs. 3.57 ± 0.23 μmol/L) compared with the control group (*P < 0.05).

In the combination groups, co-administration of amlodipine with cyclophosphamide resulted in a significant reduction in GGT activity compared to cyclo alone (0.81 ± 0.11 vs. 1.32 ± 0.34 U/g; #P < 0.05), accompanied

Table[2]: Effects of Cyclo, Amlo, and Aceta, administered individually or in combination, on renal GGT activity and oxidative stress markers (MDA and NO).

Groups/ Tests	GGT (U/g)	MDA (nmol/g)	NO (μmol/L)
(G1) Control	0.80 ± 0.16	31.37 ± 10.05	3.57 ± 0.23
(G2) Cyclo	$1.32 \pm 0.34^{*}$	$70.42 \pm 10.38^{*}$	3.09 ± 0.33
(G3) Amlo	$0.71 \pm 0.09^{\#}$	$27.19 \pm 13.14^{\#}$	3.52 ± 0.54
(G4) Aceta	$1.36 \pm 0.18^{*}$	$128.4 \pm 22.11^{*/\#}$	$2.75 \pm 0.72^{*}$
(G5) Cyclo + Amlo	$0.81 \pm 0.11^{\#}$	51.06 ± 15.71	3.55 ± 0.22
(G6) Cyclo + Aceta	$1.61 \pm 0.30^{*}$	$111.60 \pm 9.45^{*/\#}$	$2.99 \pm 0.18^{*}$
(G7) Cyclo + Amlo + Aceta	$0.81 \pm 0.16^{\#}$	$81.4 \pm 13.06^{*}$	3.29 ± 0.31

► *Data expressed as mean ± SD. Statistical analysis carried out by Ordinary one − way ANOVA followed by Tukey multiple comparisons test.*
►$*$*Significantly different with respect to control* ($P < 0.05$), *#Significantly different with respect to cyclophosphamide* ($P < 0.05$), *Gamma−glutamyl transpeptidase* (*GGT*), *Malondialdehyde* (*MDA*), *and Nitric oxide* (*NO*).

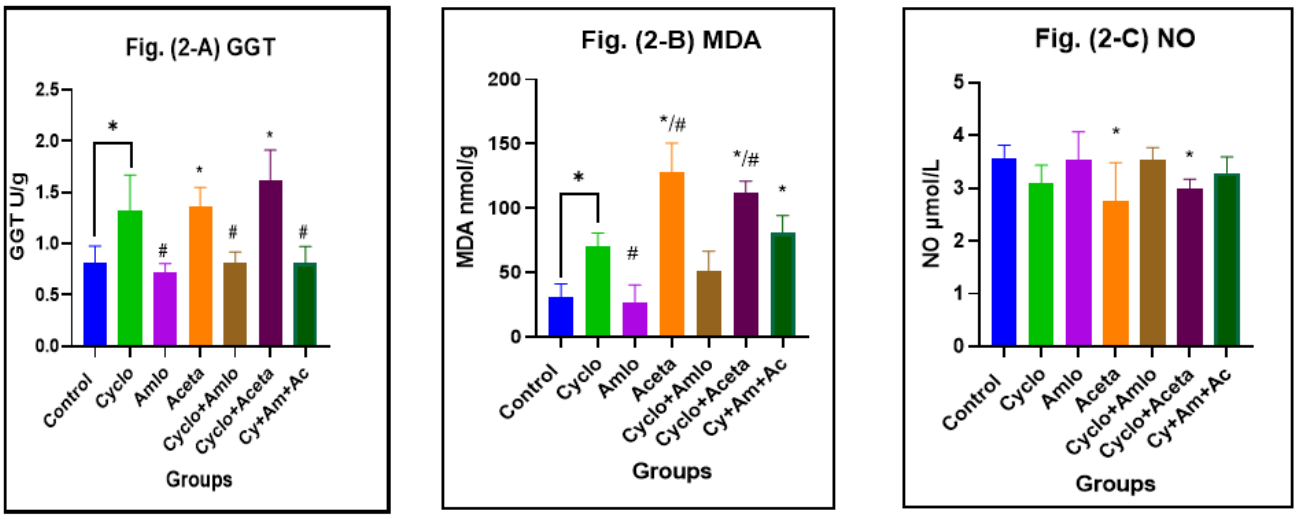

Figure 2. (A,B,C) Effects of Cyclo, Amlo, and Aceta, administered individually or in combination, on renal GGT activity and oxidative stress markers (MDA and NO).

by a non-significant decrease in MDA levels (51.06 ± 15.71 vs. 70.42 ± 10.38 nmol/g) and partial restoration of NO levels (3.55 ± 0.22 vs. 3.09 ± 0.33 μmol/L). In contrast, the combination of cyclophosphamide and acetaminophen markedly exacerbated oxidative stress, as reflected by significantly elevated GGT activity (1.61 ± 0.30 vs. 0.80 ± 0.16 U/g) and MDA levels (111.60 ± 9.45 vs. 31.37 ± 10.05 nmol/g), along with significantly reduced NO levels (2.99 ± 0.18 vs. 3.57 ± 0.23 μmol/L) compared with the control group (*P < 0.05).

Notably, the triple combination (Cyclo + Amlo + Aceta) normalized GGT activity relative to Cyclo-treated animals (0.81 ± 0.16 vs. 1.32 ± 0.34 U/g; #P < 0.05) and maintained NO levels (3.29 ± 0.31 μmol/L) without significant deviation from the control values, although MDA levels remained significantly elevated compared with the control group (81.4 ± 13.06 vs. 31.37 ± 10.05 nmol/g; *P < 0.05).

Consistent with the antioxidant status, Table (3) shows that cyclophosphamide (Cyclo) administration resulted in a significant impairment of the renal antioxidant defense system, as evidenced by marked reductions in reduced glutathione (GSH), glutathione peroxidase (GPx), catalase (CAT), and superoxide dismutase (SOD) levels compared to the control group. Specifically, GSH levels decreased from (26.50 ± 6.48) to (17.52 ± 4.94 mg/g), GPx activity declined from (27.00 ± 6.21) to (19.35 ± 1.06 U/g), CAT activity was reduced from (10.25 ± 0.45) to (4.33 ± 0.56 U/g), and SOD activity decreased from (451.6 ± 21.39) to (353.8 ± 74.87 U/g) (P < 0.05).

Administration of amlodipine (Amlo) alone did not compromise antioxidant capacity; rather, it maintained or enhanced antioxidant enzyme activities relative to the Cyclo group, as reflected by increased GSH (28.64 ± 7.40 mg/g), GPx (27.55 ± 4.28 U/g), CAT (13.75 ± 1.37 U/g), and SOD levels (483.7 ± 55.60 U/g), with CAT activity showing a significant increase compared with the control and Cyclo groups (*P < 0.05, #P < 0.05).

In the combination groups, co-administration of

Table[3]: Effects of Cyclo, Amlo, and Aceta individually or in combination on GSH, GPx, CAT, NO, and SOD antioxidant on kidney tissue.

Groups/ Tests	GSH mg/g	GPX U/g	CAT U/g	SOD U/g
(G1) Control	26.50 ± 6.48	27.00 ± 6.21	10.25 ± 0.45	451.6 ± 21.39
(G2) Cyclo	$17.52 \pm 4.94^{*}$	$19.35 \pm 1.06^{*}$	$4.33 \pm 0.56^{*}$	$353.8 \pm 74.87^{*}$
(G3) Amlo	$28.64 \pm 7.40^{\#}$	$27.55 \pm 4.28^{\#}$	$13.75 \pm 1.37^{*/\#}$	$483.7 \pm 55.60^{\#}$
(G4) Aceta	$16.99 \pm 5.07^{*}$	22.34 ± 1.58	$7.17 \pm 0.45^{*/\#}$	$340.7 \pm 45.00^{*}$
(G5) Cyclo + Amlo	22.41 ± 3.47	23.19 ± 1.30	$6.75 \pm 0.74^{*/\#}$	$489.6 \pm 68.85^{\#}$
(G6) Cyclo + Aceta	$17.03 \pm 3.52^{*}$	$17.95 \pm 1.83^{*}$	$3.20 \pm 0.49^{*}$	$328.2 \pm 26.99^{*}$
(G7) Cyclo + Amlo + Aceta	$15.97 \pm 4.89^{*}$	$19.11 \pm 3.01^{*}$	$3.01 \pm 0.23^{*}$	417.0 ± 22.15

► *Data expressed as mean ± SD. Statistical analysis carried out by Ordinary one − way ANOVA followed by Tukey multiple comparisons test.*
►$*$*Significantly different with respect to control* ($P < 0.05$), *#Significantly different with respect to cyclophosphamide* ($P < 0.05$), *Glutathione reduced* (GSH), *Glutathione peroxidase* ($GPxs$), *Reduced Catalase* (CAT), *Superoxide Dismutase* (SOD).

amlodipine with cyclophosphamide partially attenuated cyclophosphamide-induced antioxidant depletion.

This effect was evidenced by higher GSH levels (22.41 ± 3.47 mg/g) and significantly increased SOD activity (489.6 ± 68.85 U/g) compared with the Cyclo group (#P < 0.05). However, CAT activity remained significantly reduced (6.75 ± 0.74 U/g; *P < 0.05), and GPx activity (23.19 ± 1.30 U/g) did not differ significantly between the control and Cyclo groups.

In contrast, co-administration of acetaminophen with cyclophosphamide sustained the significant suppression of antioxidant markers observed with cyclo treatment alone, with reduced GSH (17.03 ± 3.52 mg/g), GPx activity (17.95 ± 1.83 U/g), CAT activity (3.20 ± 0.49 U/g), and SOD levels (328.2 ± 26.99 U/g), all of which were significantly lower than the control values (*P < 0.05).

The triple combination (Cyclo + Amlo + Aceta) did not fully restore the renal antioxidant capacity, as GSH (15.97 ± 4.89 mg/g), GPx (19.11 ± 3.01 U/g), and CAT activity (3.01 ± 0.23 U/g) remained significantly reduced compared with the control group (*P < 0.05). Nevertheless, SOD levels (417.0 ± 22.15 U/g) were higher than those observed in the cyclo group, although this increase did not reach statistical significance.

Histopathological evaluation of kidney tissues (Table 4) revealed normal renal architecture in the control group (G1), characterized by intact glomeruli and renal tubules with no evidence of tubular necrosis, tubular dilatation, inflammatory cell infiltration, glomerular shrinkage, or tubular atrophy. Similarly, kidneys from the amlodipine (Amlo)-treated group exhibited preserved histological features, indicating the absence of intrinsic renal toxicity.

In contrast, cyclophosphamide (Cyclo)-treated rats showed marked renal injury, as evidenced by moderate tubular necrosis (++), tubular dilatation (++), loss of the brush border in focal tubules (++), severe focal inflammatory cell infiltration (+++), glomerular shrinkage (++), and tubular atrophy (++). These findings confirm the pronounced nephrotoxic effects of cyclophosphamide at the histological level.

Acetaminophen (Aceta) administration alone induced mild renal alterations, including tubular dilatation (+), focal loss of the brush border (+), inflammatory cell infiltration (+), and tubular atrophy (+). Tubular necrosis and glomerular shrinkage were absent, suggesting mild tubular stress without overt structural damage.

In the combination groups, co-administration of amlodipine with cyclophosphamide markedly attenuated cyclophosphamide-induced renal damage, with most histopathological parameters showing minimal to absent changes, including the absence of tubular necrosis, tubular dilatation, inflammatory infiltration, and glomerular shrinkage, indicating a clear protective effect of amlodipine against cyclophosphamide-induced nephrotoxicity.

Conversely, the combination of cyclophosphamide and acetaminophen resulted in severe histological deterioration, characterized by extensive tubular necrosis (+++), marked tubular dilatation (++), severe loss of the brush border (+++), pronounced inflammatory cell infiltration (+++), significant glomerular shrinkage (+++), and tubular atrophy (++), indicating the exacerbation of cyclophosphamide-induced renal injury.

Notably, the triple combination (Cyclo + Amlo + Aceta) demonstrated substantial histological improvement compared with the Cyclo + Aceta group, with only mild-to-moderate changes observed across most parameters, reflecting the protective influence of amlodipine even in the presence of acetaminophen.

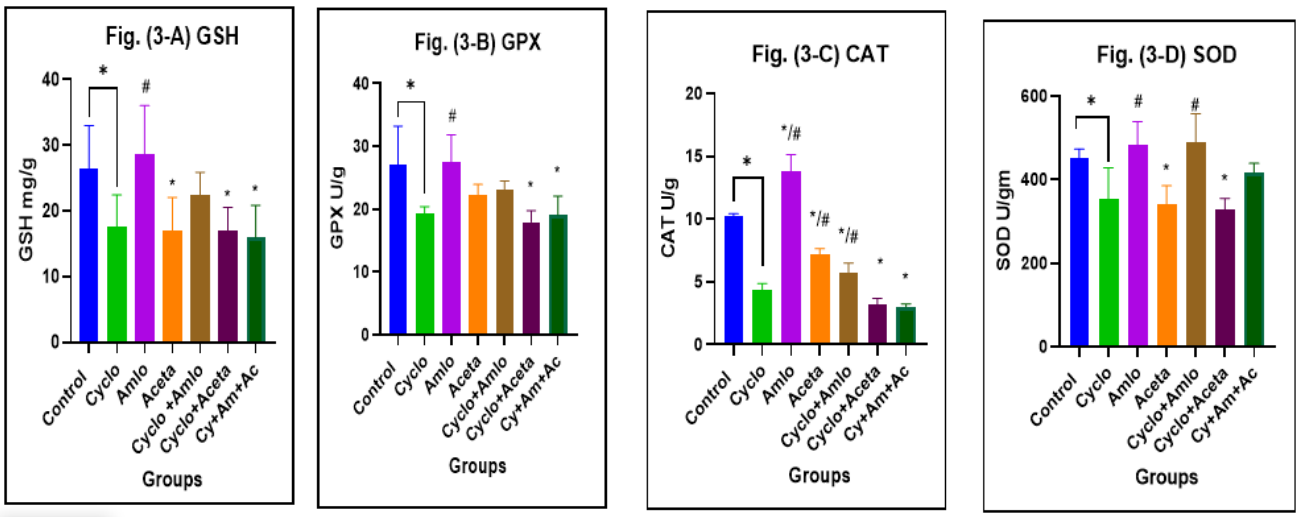

Figure 3. (A,B,C) Effects of Cyclo, Amlo, and Aceta individually or in combination on GSH, GPx, CAT, NO, and SOD antioxidant on kidney tissue.

Table[4]: Histopathological changes in kidney tissues across various experimental groups.

Histopathological findings	G1	Cyclo	Amlo	Aceta	Amlo + Cyclo	Aceta + Cyclo	Amlo + Aceta + Cyclo
Tubular necrosis	-	++	-	-	-	+++	+
Tubular dilatation	-	++	-	+	-	++	+
Loss of the brush border in focal tubules	-	++	-	+	+	+++	+
Focal inflammatory cells infiltration	-	+++	-	+	-	+++	++
Glomerular shrinkage	-	++	-	-	-	+++	++
Tubular atrophy	-	++	-	+	+	++	+

► $+++Acute\ (75-100\%),\ ++\ Moderate\ (50-75\%),\ +\ Mild\ (25-50\%),\ -\ Nil\ (0-25\%).$

Consistent with the histopathological findings, quantitative morphometric analysis of the glomerular capsular space (GCS) (Table 5) showed a significant enlargement in the Cyclo-treated group (32.7 ± 15.5 μm) compared with the control group (12.13 ± 5.5 μm; *P = 0.0038), reflecting glomerular shrinkage and structural damage. Treatment with amlodipine or acetaminophen alone did not result in significant changes in the GCS compared to the control group.

Co-administration of amlodipine with cyclophosphamide markedly reduced GCS enlargement (16.5 ± 5.8 μm), approaching control values and showing a near-significant difference compared with the Cyclo group (#P = 0.0516). In contrast, the Cyclo + Aceta group exhibited persistently enlarged GCS values (32.8 ± 8.2 μm), comparable to those of the Cyclo group, indicating sustained glomerular injury.

Importantly, the triple combination (cyclosporine + amlodipine + acetylcysteine) resulted in a significant reduction in GCS (16.8 ± 4.3 μm) compared with the cyclosporine + acetylcysteine group (P = 0.0073), further supporting the histological evidence of amlodipine-mediated renoprotection.

Collectively, the histopathological and morphometric findings demonstrate that cyclophosphamide induces pronounced renal structural damage, which is markedly aggravated by acetaminophen co-administration, whereas amlodipine exerts a clear renoprotective effect by preserving tubular integrity and glomerular architecture.

Measurement of histopathological examinations of kidney tissue.

Kidney histopathological examinations were performed to evaluate the structural changes and the extent of tissue damage or recovery, as follows:

1) Control (G1):

Table[5]: Effects of Amlo and or/ Aceta on (GCS) in Cyclo-induced renal toxicity in rat.

Groups	Glomeruli capsular spaces (μm)			
	Mean ± SD	[Min; Max]	SEM	*P.* Value
(G1) Control	12.13 ± 5.5	[8;25]	1.9	–
(G2) Cyclo	32.7 ± 15.5	[14;56]	5.4	0.0038^{*}
(G3) Amlo	14.0 ± 7.2	[7;12]	0.64	$> 0.9999^{*}$
(G4) Aceta	24.4 ± 7.8	[12;37]	2.7	0.3477^{*}
(G5) Cyclo + Amlo	16.5 ± 5.8	[7;24]	2.1	$0.0516^{\#}$
(G6) Cyclo + Aceta	32.8 ± 8.2	[22;47]	2.9	$> 0.9999^{\#}$
(G7) Cyclo + Amlo + Aceta	16.8 ± 4.3	[10;23]	1.5	$0.0073^{\#}$

► *Results are presented as the mean* $\pm$ *SD* $(n = 8)$.
► *One* $-$ *way ANOVA followed by Tukey's multiple comparisons test, were used for statistical analysis,* $*p < 0.05$.
► *Periodic acid* $-$ *Schiff* $-$ *stained images of kidney cortex under* 400 *x magnification showed shrunken were measured by calculation of used Olympus Microscope BX*53.
► $* = Control\ vs.\ Group;\ \# = Cyclo\ vs.\ Group.$

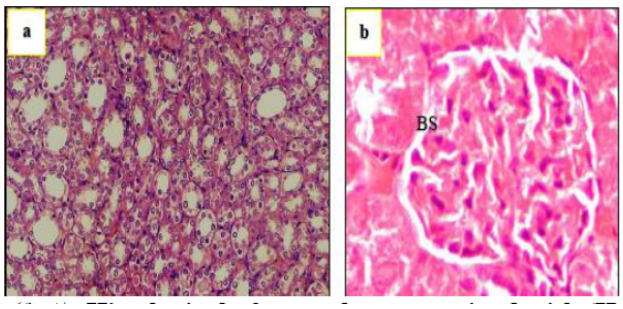

figure 1-A Histological of control group stained with (H&E).

Histological photomicrographs of kidney sections from normal rats showed normal cortical and medullary architecture, with intact renal tubules and normally spaced Bowman's capsules.

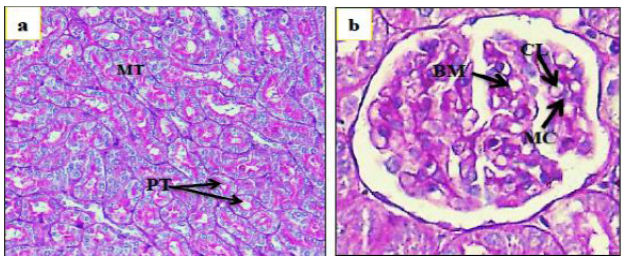

figure 1-B Histological of control group stained with (PAS).

PAS-stained kidney sections from normal rats revealed intact proximal and medullary tubules with distinct brush borders, along with normal glomeruli exhibiting well-defined capillary loops, thin basement membranes, and normally sized mesangial cells.

2) Cyclophosphamide (G2):

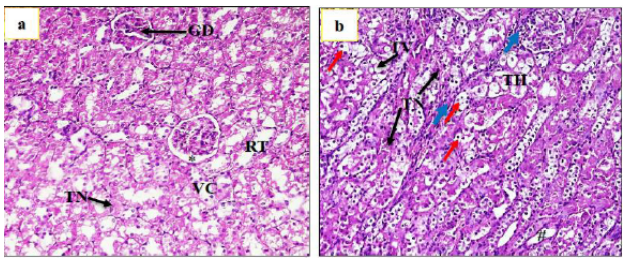

figure 2-A Histological of Cyclophosphamide group stained with (H&E).

Histological photomicrographs (200×, H&E staining) of renal sections from cyclo-treated rats showed marked glomerular degeneration (GD), widening of Bowman's space (*), tubular congestion, cytoplasmic vacuolation (VC), and acute tubular necrosis (TN) compared with the normal control group. Scattered necrotic and regenerating tubules (RT) were also observed. The proximal tubules exhibited hydropic degeneration (TH), tubular necrosis (TN), and pronounced tubulointerstitial inflammation (blue arrows), along with numerous apoptotic tubular cells (red arrows).

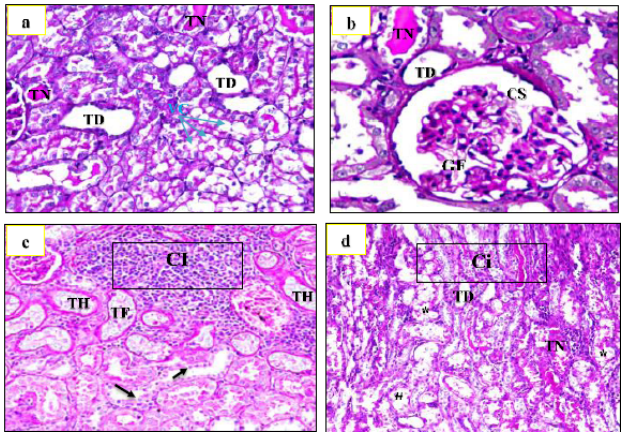

figure 2-B Histological of Cyclophosphamide group stained with (PAS).

PAS-stained kidney sections from cyclophosphamide-treated rats showed pronounced glomerular fibrosis (GF), tubular dilatation (TD), and tubular injury characterized by cellular congestion, cytoplasmic vacuolation (VC), tubular necrosis (TN), and widening of the capsular space [(a) 200×, (b) 400×].

Panel (c) shows acute inflammatory cell infiltration (CI), attenuation of tubular epithelial cells with increased eosinophilic staining, tubular fibrosis (TF), hydropic degeneration (TH), and loss of the brush border (black arrow) (200×).

Panel (d) shows tubular duct hypertrophy (*), necrosis (TN), regeneration of tubular structures (#), cytoplasmic vacuolation (black arrow), and inflammatory cell infiltration (CI) (200×).

3) Acetaminophen (G3):

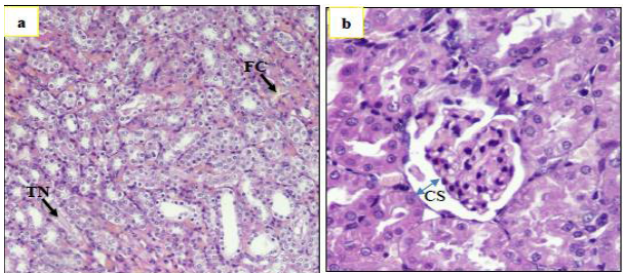

figure 3-A Histological of Acetaminophen group stained with (H&E).

H&E-stained kidney sections from acetaminophen-treated rats showed tubular attenuation with cell sloughing, fibrin casts, renal tubular necrosis, and increased capsular space compared to the controls.

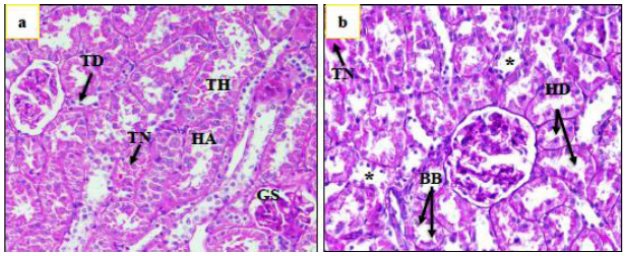

figure 3-B Histological of Acetaminophen group stained with (PAS).

PAS-stained kidney sections from acetaminophen-treated rats showed tubular hydropic changes, dilatation, necrosis, hyperatrophy, glomerular shrinkage, loss of brush borders, and focal tubular regeneration.

4) Amlodipine (G4):

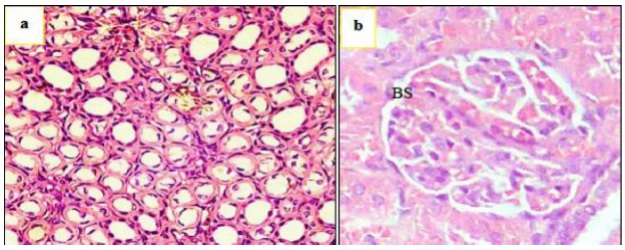

figure 4-A Histological of Amlodipine group stained with (H&E).

H&E-stained kidney sections from amlodipine-treated rats showed a normal cortex, intact medullary tubules, normal capillary loops, mesangial regions, and Bowman's space.

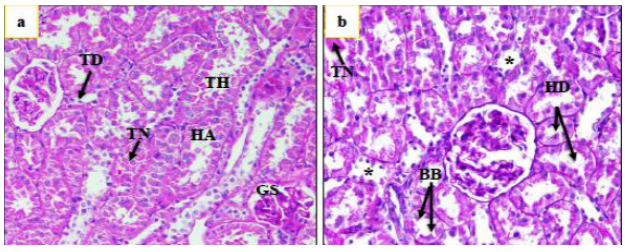

figure 4-B Histological of Amlodipine group stained with (PAS).

PAS-stained kidney sections from amlodipine-treated rats showed a normal renal cortex with intact proximal tubules and brush borders, as well as normal glomeruli and Bowman's space.

5) Cyclophosphamide + Amlodipine (G5):

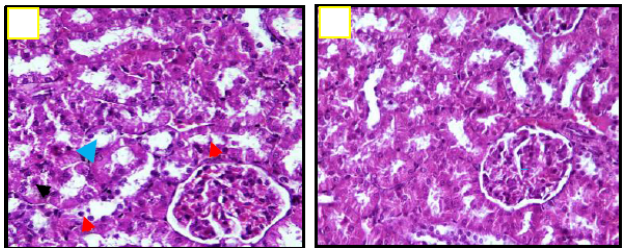

figure 5-A Histological of Cyclophosphamide + Amlodipine group stained with (H&E).

Histological examination of kidney sections from rats treated with amlodipine in combination with cyclophosphamide (H&E, ×400) revealed a marked improvement in renal architecture compared with the cyclophosphamide-only group. (a) Kidney sections showing reduced tubular degeneration and dilatation, with minimal tubular necrosis (black arrow) and few vacuolated tubular epithelial cells (red arrow). (b) The proximal tubules appeared nearly normal, with preserved glomerular structure (G) and reduced Bowman's space (BC), indicating attenuation of cyclo-induced renal injury.

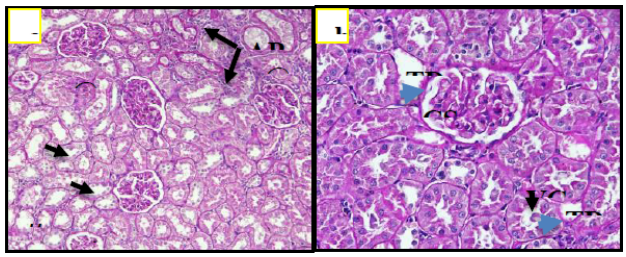

figure 5-B Histological of Cyclophosphamide + Amlodipine group stained with (PAS).

Histological photomicrographs (PAS staining) of kidney sections from rats treated with Amlo and Cyclo showed partial restoration of renal histoarchitecture.

(a) Sections exhibited moderate focal loss of the tubular brush border (black arrow), mild pyelonephritis, limited glomerular shrinkage (*), and mild tubular dilatation and necrosis (200×).

(b) A reduction in Bowman's capsule space (CS), moderate cytoplasmic vacuolation (VC), and thickening of the tubular basement membrane with mild tubular dilatation (blue arrow) were also observed (400×).

6) Cyclophosphamide + Acetaminophen (G6):

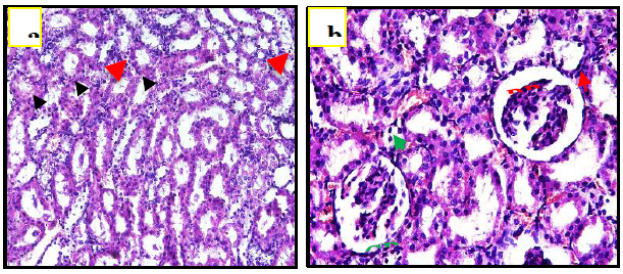

figure 6-A Histological of Cyclophosphamide + Acetaminophen group stained with (H&E).

Histological photomicrographs (H&E staining) of kidney sections from rats co-treated with acetaminophen and cyclophosphamide demonstrated aggravated renal damage.

(a) Proximal tubules exhibited acute tubular necrosis (arrowheads), tubular dilatation (TD), and cytoplasmic vacuolation (red arrow) (magnification, 200×).

(b) Renal corpuscles showing dilated Bowman's space (DB), glomerular degeneration (GD), tubular necrosis (blue arrow), and pronounced tubulointerstitial inflammation (red arrow) accompanied by apoptotic tubular cells (green arrow) (400×). These alterations suggest that Aceta co-administration intensified cyclo-induced renal injury.

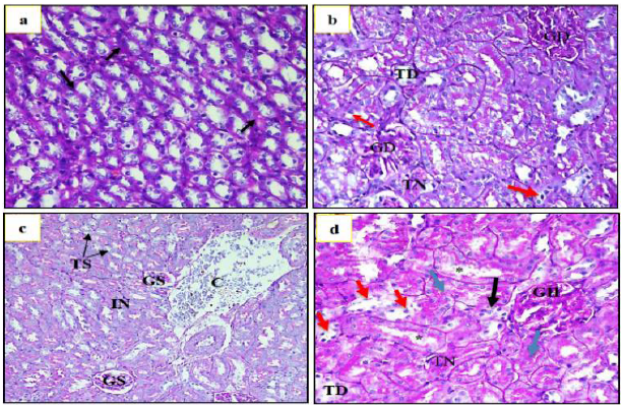

figure 6-B Histological of Cyclophosphamide + Acetaminophen group stained with (PAS).

PAS-stained kidney sections (4 μm) from rats treated with Aceta + Cyclo revealed severe histopathological alterations.

(a) Tubular shrinkage with thickened basement membranes (black arrow) (magnification 400×).

(b) Marked glomerular degeneration (GD), tubular dilatation (TD), necrosis (TN), and cytoplasmic vacuolation (red arrow) (magnification 200×).

(c) Evident glomerular and tubular shrinkage (GS, TS), vascular congestion (C), and inflammatory cell infiltration (IN) (magnification 200×).

(d) Pronounced cytoplasmic vacuolation (black arrow), tubular necrosis (blue arrow), glomerular hemorrhage

(GH), dilated tubules (TD), tubular atrophy (*), and karyolysis (blue arrow) (400×).

7) Cyclophosphamide + Amlodipine + Acetaminophen (G7):

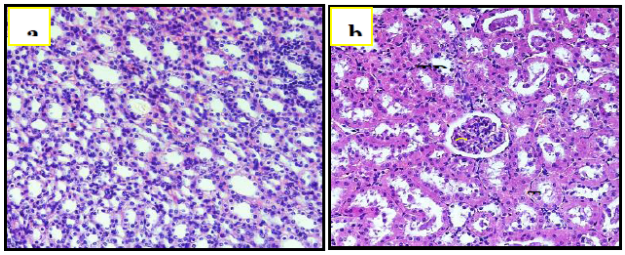

figure 7-A Histological of Cyclophosphamide + Amlodipine + Acetaminophen group stained with (H&E).

H&E-stained kidney sections from rats treated with cyclo + amlo + acet showed marked histological improvement.
(a) Reduced tubular damage and dilatation in the renal medulla (200×).
(b) Nearly normal glomeruli with narrowed Bowman's space (arrow), intact renal tubules (T), and minimal tubular degeneration (TD) with occasional casts (*) (magnification 200×).

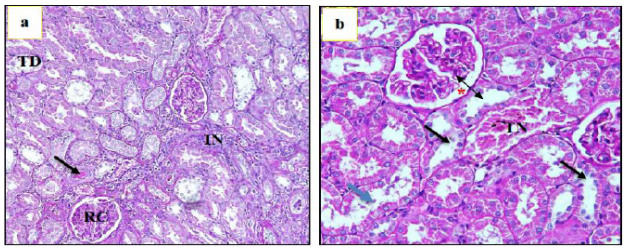

figure 7-B Histological Cyclophosphamide + Amlodipine + Acetaminophen group stained with (PAS).

PAS-stained kidney sections from rats treated with amlo + acet + cyclo showed marked histological recovery.
(a) Reduced tubular dilatation (TD), normal renal corpuscles (RC), and mild tubular shrinkage with limited inflammatory infiltration (IN)(200×).
(b) Nearly normal capsular space (*), mild tubular necrosis (TN), slight vacuolation, focal tubular atrophy (blue arrow), and moderate brush border loss (black arrow) (magnification 400×).

4. DISCUSSION

This study evaluated cyclophosphamide (Cyclo)-induced nephrotoxicity and the modulatory effects of amlodipine (Amlo) and acetaminophen (Aceta) using integrated functional, biochemical, and histopathological analyses.

Cyclo administration did not significantly alter conventional renal function markers (BUN, urea, and serum creatinine), suggesting an apparent absence of overt renal dysfunction in the rats. However, biochemical and histological findings revealed clear evidence of injuries. Cyclo markedly elevated -glutamyl transpeptidase (GGT) and malondialdehyde (MDA) levels while significantly depleting antioxidant defenses (GSH, GPx, CAT, and SOD), indicating pronounced oxidative stress [43–45]. These results are consistent with those of previous studies [27, 46, 47]. Histopathological examination confirmed glomerular degeneration, tubular necrosis, and inflammatory infiltration [43, 44], highlighting early renal injury despite near-normal functional markers of renal injury.

Co-administration of acetaminophen with cyclophosphamide further exacerbated oxidative stress and structural damage, demonstrating a synergistic nephrotoxic effect [48, 49]. This is likely mediated by the reactive metabolite NAPQI and associated with glutathione depletion [50, 51].

In contrast, amlo significantly attenuated oxidative stress, preserved antioxidant defenses, and improved renal histoarchitecture, consistent with its established antioxidant properties [24].

Notably, nitric oxide levels, which were reduced by Cyclo and Aceta, were maintained with amlo, suggesting an improved redox balance.

Combined administration of Amlo and Aceta with Cyclo provided partial protection, with moderate improvement compared to the cyclo–aceta group, though not complete normalization. Mechanistically, Cyclo-induced toxicity is attributed to acrolein-mediated ROS generation, lipid peroxidation, and GSH depletion [16, 18], whereas GGT activity may further amplify oxidative injury [52]. Amlo counteracts these effects by enhancing antioxidant capacity and stabilizing redox homeostasis [24].

Overall, these findings demonstrate that cyclo induces early renal injury that is not adequately detected using conventional renal function markers. Oxidative stress biomarkers and histopathological alterations provide more sensitive indicators of nephrotoxicity, underscoring the importance of integrating biochemical and structural assessments in the evaluation of drug-induced renal injury.

5. CONCLUSION

Cyclophosphamide induces significant renal injury that is not adequately detected by conventional renal function markers but is clearly evident at the oxidative and histopathological levels. Amlodipine exerts a protective

effect by attenuating oxidative stress and preserving renal structure, whereas acetaminophen aggravates renal injury and enhances the oxidative burden.

Accordingly, nephrotoxicity evaluation should include oxidative stress biomarkers in addition to routine renal function tests. Furthermore, co-administration of amlodipine with cyclophosphamide may offer a protective strategy against renal damage, whereas the use of acetaminophen in combination with cyclophosphamide should be approached with caution because of its potential to exacerbate nephrotoxicity.

REFERENCES

[1] M. G. Neuman, S. Malnick, and L. Chertin, "Gamma glutamyl transferase–an underestimated marker for cardiovascular disease and the metabolic syndrome," *J. Pharm. & Pharm. Sci.*, vol. 23, pp. 65–74, 2020.

[2] Y. Chang et al., "Nonalcoholic fatty liver disease predicts chronic kidney disease in nonhypertensive and nondiabetic korean men," *Metabolism*, vol. 57, no. 4, pp. 569–576, 2008.

[3] I. Lippi et al., "Clinical utility of urine kidney injury molecule-1 (kim-1) and gamma-glutamyl transferase (ggt) in the diagnosis of canine acute kidney injury," *Vet. Res. Commun.*, vol. 42, no. 2, pp. 95–100, 2018.

[4] M. H. Rosner and M. A. Perazella, "Acute kidney injury in patients with cancer," *New Engl. J. Med.*, vol. 376, no. 18, pp. 1770–1781, 2017.

[5] L. Awdishu and R. L. Mehta, "The 6r's of drug induced nephrotoxicity," *BMC Nephrol.*, vol. 18, no. 1, p. 124, 2017.

[6] C. A. Naughton, "Drug-induced nephrotoxicity," *Am. Fam. Physician*, vol. 78, no. 6, pp. 743–750, 2008.

[7] R. D. Beger, J. Sun, and L. K. Schnackenberg, "Metabolomics approaches for discovering biomarkers of drug-induced hepatotoxicity and nephrotoxicity," *Toxicol. Appl. Pharmacol.*, vol. 243, no. 2, pp. 154–166, 2010.

[8] W. S. Waring and A. Moonie, "Earlier recognition of nephrotoxicity using novel biomarkers of acute kidney injury," *Clin. Toxicol.*, vol. 49, no. 8, pp. 720–728, 2011.

[9] E. J. Weber, J. Himmelfarb, and E. J. Kelly, "Concise review: Current and emerging biomarkers of nephrotoxicity," *Curr. Opin. Toxicol.*, vol. 4, pp. 16–21, 2017.

[10] M. A. Ayza et al., "Potential protective effects of antioxidants against cyclophosphamide-induced nephrotoxicity," *Int. J. Nephrol.*, vol. 2022, no. 1, p. 5 096 825, 2022.

[11] A. S. Al-Homsi et al., "Post-transplant high-dose cyclophosphamide for the prevention of graft-versus-host disease," *Biol. Blood Marrow Transplant.*, vol. 21, no. 4, pp. 604–611, 2015.

[12] M. Ahlmann and G. Hempel, "The effect of cyclophosphamide on the immune system: Implications for clinical cancer therapy," *Cancer Chemother. Pharmacol.*, vol. 78, no. 4, pp. 661–671, 2016.

[13] F. Gagne, *Biochemical Ecotoxicology: Principles and Methods*. Elsevier, 2014.

[14] A. Stankiewicz and E. Skrzydlewska, "Protection against cyclophosphamide-induced renal oxidative stress by amifostine: The role of antioxidative mechanisms," *Toxicol. Mech. Methods*, vol. 13, no. 4, pp. 301–308, 2003.

[15] X.-M. Meng, D. J. Nikolic-Paterson, and H. Y. Lan, "Inflammatory processes in renal fibrosis," *Nat. Rev. Nephrol.*, vol. 10, no. 9, pp. 493–503, 2014.

[16] P. Abraham and B. Isaac, "The effects of oral glutamine on cyclophosphamide-induced nephrotoxicity in rats," *Hum. & Exp. Toxicol.*, vol. 30, no. 7, pp. 616–623, 2011.

[17] H. Wanas et al., "Nebivolol protects against cyclophosphamide-induced nephrotoxicity through modulation of oxidative stress, inflammation, and apoptosis," *Clin. Exp. Pharmacol. Physiol.*, vol. 48, no. 5, pp. 811–819, 2021.

[18] T. Uehara et al., "Comparative nephrotoxicity of cisplatin and nedaplatin: Mechanisms and histopathological characteristics," *J. Toxicol. Pathol.*, vol. 24, no. 2, pp. 87–94, 2011.

[19] A. A. Azouz, E. A.-N. Abdel-Razek, and A. M. Abo-Youssef, "Amlodipine alleviates cisplatin-induced nephrotoxicity in rats through gamma-glutamyl transpeptidase (ggt) enzyme inhibition," *Saudi Pharm. J.*, vol. 28, no. 11, pp. 1317–1325, 2020.

[20] H. S. Bhatia, D. Sharma, and S. Dandawate, "Case report: Amlodipine-induced acute kidney injury due to severe hypotension," *J. Nephrol. & Ther.*, vol. 8, no. 1, p. 298, 2018.

[21] H. Zhang and H. J. Forman, "Glutathione synthesis and its role in redox signaling," in *Seminars in Cell & Developmental Biology*, Elsevier, 2012.

[22] S. A. El-Maraghy, S. M. Rizk, and N. N. Nassar, "Protective effect of amlodipine against nephrotoxicity induced by cisplatin and gentamicin in rats," *Arch. Toxicol.*, vol. 89, no. 10, pp. 1829–1837, 2015.

[23] E. J. Park, D. H. Kim, S. H. Kim, and J. Y. Choi, "Calcium channel blockers and renal protection: Beyond blood pressure lowering," *Kidney Res. Clin. Pract.*, vol. 38, no. 1, pp. 4–14, 2019.

[24] Y. Hirooka et al., "Amlodipine-induced reduction of oxidative stress in the brain is associated with sympatho-inhibitory effects in stroke-prone spontaneously hypertensive rats," *Hypertens. Res.*, vol. 29, no. 1, pp. 49–56, 2006.

[25] H. Darvishi-Khezri et al., "Amlodipine: Can act as an antioxidant in patients with transfusion-dependent beta-thalassemia?" *J. Clin. Lab. Anal.*, vol. 36, no. 12, e24752, 2022.

[26] V. Gerriets et al., *Acetaminophen*, 2024.

[27] M. A. Ayza et al., "Protective effect of croton macrostachyus stem bark on cyclophosphamide-induced nephrotoxicity in rats," *J. Exp. Pharmacol.*, pp. 275–283, 2020.

[28] National Research Council, *Guide for the Care and Use of Laboratory Animals*, 8th ed. National Academies Press, 2011.

[29] American Veterinary Medical Association, *Avma guidelines for the euthanasia of animals*, 2020.

[30] National Research Council, *Guide for the Care and Use of Laboratory Animals*, 8th ed. Washington: National Academies Press, 2011.

[31] S. Parasuraman, R. Raveendran, and R. Kesavan, "Blood sample collection in small laboratory animals," *J. Pharmacol. & Pharmacother.*, vol. 1, no. 2, p. 87, 2010.

[32] American Veterinary Medical Association, *Avma guidelines for the euthanasia of animals*, Available online, 2020.

[33] E. Beutler, O. Duron, and M. B. Kelly, "Improved method for the determination of blood glutathione," *J. Lab. Clin. Med.*, vol. 61, pp. 882–888, 1963.

[34] D. E. Paglia and W. N. Valentine, "Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase," *J. Lab. Clin. Med.*, vol. 70, no. 1, pp. 158–169, 1967.

[35] H. Ohkawa, W. Ohishi, and K. Yagi, "Colorimetric method for determination of mda activity," *Biochemistry*, vol. 95, pp. 90 738–90 743, 1979.

[36] P. Fossati, L. Prencipe, and G. Berti, "Use of chromogenic system in direct enzymic assay of uric acid in serum and urine," *Clin. Chem.*, vol. 26, no. 2, pp. 227–231, 1980.

[37] H. Aebi, "Catalase in vitro," *Methods Enzymol.*, vol. 105, pp. 121–126, 1984.

[38] H. Montgomery and J. F. Dymock, "Analyst study," *Analyst*, vol. 86, p. 414, 1961.

[39] M. Nishikimi, N. A. Rao, and K. Yagi, "Superoxide dismutase activity study," *Biochem. Biophys. Res. Commun.*, vol. 46, pp. 849–854, 1972.

[40] E. Wehrle et al., "Protocol for preparing formalin-fixed paraffin-embedded musculoskeletal tissue samples from mice for spatial transcriptomics," *STAR Protoc.*, vol. 5, no. 2, p. 102 986, 2024.

[41] J. D. Bancroft and M. Gamble, *Theory and Practice of Histological Techniques*. Elsevier Health Sciences, 2008.

[42] F. A. Alshubaily and E. J. Jambi, "Correlation between antioxidant and anti-osteoporotic activities of shilajit loaded into chitosan nanoparticles," *Polymers*, vol. 14, no. 19, p. 3972, 2022.

[43] S. Çetik-Yıldız et al., "Immunotherapeutic and cell-protective effects of probiotic kefir on cyclophosphamide-induced nephrotoxicity," *Afyon Kocatepe Univ. J. Sci. Eng.*, vol. 24, no. 6, pp. 1275–1284, 2024.

[44] M. U. Ijaz et al., "Ameliorative effect of herbacetin against cyclophosphamide-induced nephrotoxicity in rats," *Hum. & Exp. Toxicol.*, vol. 41, p. 09 603 271 221 132 140, 2022.

[45] Y. Temel et al., "Protective effect of chrysin on cyclophosphamide-induced hepatotoxicity and nephrotoxicity," *Naunyn-Schmiedeberg's Arch. Pharmacol.*, vol. 393, no. 3, pp. 325–337, 2020.

[46] M. Cengiz et al., "Protective effect of escin against kidney injury," *Curr. Issues Mol. Biol.*, vol. 46, no. 12, pp. 13 471–13 481, 2024.

[47] A. F. Tohamy et al., "Lucrative antioxidant effect of metformin against cyclophosphamide induced nephrotoxicity," *Saudi J. Biol. Sci.*, vol. 28, no. 5, p. 2755, 2021.

[48] S. Attiyah, "The consequence of antioxidant signaling pathways on acetaminophen nephrotoxicity," *J. Pioneer. Med. Sci.*, vol. 13, pp. 34–40, 2024.

[49] A. M. Muhsin et al., "Influence of celtis occidentalis extract on acetaminophen-induced nephrotoxicity," *J. Health Rehabil. Res.*, vol. 4, no. 3, pp. 1–6, 2024.

[50] H. Jaeschke, M. R. McGill, and A. Ramachandran, "Oxidant stress, mitochondria, and cell death mechanisms in drug-induced liver injury," *Drug Metab. Rev.*, vol. 44, no. 1, pp. 88–106, 2012.

[51] A. Pompella et al., "Gamma-glutamyltransferase, redox regulation and cancer drug resistance," *Curr. Opin. Pharmacol.*, vol. 7, no. 4, pp. 360–366, 2007.

[52] M. H. Hanigan et al., "Immunolabeling of gamma-glutamyl transferase 5 in normal human tissues," *Histochem. Cell Biol.*, vol. 143, no. 5, pp. 505–515, 2015.